THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 277, No. 39, Issue of September 27, pp. 36161–36166, 2002
Printed in U.S.A.

Mutational Analysis of Human Hydroxysteroid Sulfotransferase SULT2B1 Isoforms Reveals That Exon 1B of the *SULT2B1* Gene Produces Cholesterol Sulfotransferase, whereas Exon 1A Yields Pregnenolone Sulfotransferase*

Received for publication, July 17, 2002
Published, JBC Papers in Press, July 26, 2002, DOI 10.1074/jbc.M207165200

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As a result of an alternative exon 1, the gene for human hydroxysteroid sulfotransferase (*SULTB1*) encodes for two peptides differing only at their amino termini. The SULT2B1b isoform preferentially sulfonates cholesterol. Conversely, the SULT2B1a isoform avidly sulfonates pregnenolone but not cholesterol. The outstanding structural feature that distinguishes the SULT2B1 isoforms from the prototypical SULT2A1 isozyme is the presence of extended amino- and carboxyl-terminal ends in the former. Investigating the functional significance of this unique characteristic reveals that removal of 53 amino acids from the relatively long carboxyl-terminal end that is common to both SULT2B1 isoforms has no effect on the catalytic activity of either isoform. On the other hand, removal of 23 amino acids from the amino-terminal end that is unique to SULT2B1b results in loss of cholesterol sulfotransferase activity, whereas removal of 8 amino acids from the amino-terminal end that is unique to SULT2B1a has no effect on pregnenolone sulfotransferase activity. Deletion analysis along with site-directed mutagenesis of SULT2B1b reveal that the amino acid segment 19–23 residues from the amino terminus and particularly isoleucines at positions 21 and 23 are crucial for cholesterol catalysis. In the gene for *SULT2B1*, exon 1B encodes for only the unique amino-terminal region of SULT2B1b; however, exon 1A encodes for the unique amino-terminal end of SULT2B1a plus an additional 48 amino acids. Thus, if the gene for *SULT2B1* employs exon 1B, cholesterol sulfotransferase is synthesized, whereas if exon 1A is used, pregnenolone sulfotransferase is produced.

The cloning of a novel hydroxysteroid sulfotransferase (SULT)[1] subfamily in human (1) and mouse (2) species has been a significant development in the field of cytosolic sulfotransferases (3, 4). The gene for the novel human hydroxysteroid sulfotransferase (*SULT2B1*) maps to chromosome 19q13.4, which is ~500 kb telomeric to the location of the prototypical hydroxysteroid sulfotransferase gene *SULT2A1* (5). It should be noted, however, that the gene for *SULT2A1* encodes for a single polypeptide, whereas the *SULT2B1* gene, as a result of an alternative exon 1, encodes for two subtypes differing only at their amino-terminal ends. Thus, the two *SULT* genes produce three functionally related polypeptides, the physiologic significance of which is not entirely understood. SULT2A1, which is commonly referred to as dehydroepiandrosterone sulfotransferase, has a broad substrate predilection involving, in addition to dehydroepiandrosterone, a variety of neutral steroids including androgenic steroids. It also sulfonates estrogenic steroids as well as bile acids (6–9); it does not, however, sulfonate cholesterol (10). The SULT2B1 isoforms, in contrast to the SULT2A1 isozyme, have a more selective substrate preference and will not, to all intents and purposes, sulfonate testosterone, estradiol, or bile acids (10–12). Notably, the SULT2B1 isoforms, particularly SULT2B1b, sulfonate cholesterol (10), and although a clear functional difference between the SULT2B1 isoforms is now emerging, a physiologic distinction between them is not well understood.

From a structural point of view, the outstanding feature of the SULT2B1 isoforms, as compared with the SULT2A1 isozyme as well as other cloned steroid and cognate cytosolic sulfotransferases, is their extended amino- and carboxyl-terminal ends. This characteristic intrigued us as to whether there might be functional effects ascribable to either the carboxyl-terminal ends, which are structurally common to the two proteins, and/or the amino-terminal ends, which are structurally unique. The question was, therefore, posed: do either the amino- or the carboxyl-terminal ends of the human SULT2B1 isoforms have an influence on substrate specificity and/or catalysis? Interestingly, the results of these experiments reveal the selective importance of the unique amino-terminal end of the SULT2B1b isoform for both substrate specificity and catalysis. This is in contrast to the unique amino-terminal end of the SULT2B1a isoform, which neither influences substrate specificity nor is required for catalysis.

EXPERIMENTAL PROCEDURES

Materials—All steroids, sterols, 2-hydroxypropyl-β-cyclodextrin, and 3′-phosphoadenosine 5′-phosphosulfate (PAPS) were obtained from Sigma. [^{3}H]cholesterol (60 Ci/mmol) and [^{3}H]pregnenolone (17.5 Ci/mmol) were purchased from PerkinElmer Life Sciences. Silica gel TLC plates were obtained from Analtech (Newark, MA). Organic solvents were purchased from J. T. Baker Inc. and Mallinckrodt.

Expression and Purification of Human SULT2B1a and SULT2B1b—Prokaryotic expression vectors for human SULT2B1a (GenBank™ accession no. U92314) and SULT2B1b (GenBank™ accession no. U92315) were obtained using PCR at *Sal*I and *Not*I restriction sites of pGEX-6P-3 from Amersham Biosciences. Recombinant SULT2B1 subtypes were purified using the GST gene fusion system (Amersham Biosciences) according to a previously reported procedure (13). Eluates

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[1] The abbreviations used are: SULT, sulfotransferase; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; RT, reverse transcription; GST, glutathione *S*-transferase.

TABLE I

Oligonucleotide primers used in mutagenesis of human SULT2B1 isoforms

Human SULT2B1a had 52 amino acids removed from the carboxyterminus (COOH) and 8 amino acids removed from the amino terminus (NH_3): Human SULT2B1b also had 52 amino acids removed from the carboxyl terminus as well as 8, 18 and 23 amino acids removed from the aminoterminus. In addition, point mutations of a DISEI amino acid sequence (residues 19–23) in the aminoterminal region of SULT2B1b were carried out (compare Fig. 2). Underlined nucleotides indicate *Sal*I and *Not*I restriction sites.

Construct	Sense/Antisense	Primers
SULT2B1a		
1. COOH	S	3′-GTCGACATGGCGTCTCCCCCACCTTTCCAC-3′
	AS	5′-AGCGGCCGCTTAGTCTTCATCCCAGGGGAAGGTCGGCATC-3′
2. NH_3	S	5′-ACGCGTCGACAGCCAGAAGTTGCCAGGTGAATAC-3′
	AS	5′-GCGGCCGCTTATTATGAGGGTCGTGGGTGCGG-3′
SULT2B1b		
1. COOH	S	5′-GTCGACATGGACGGGCCCGCCGAGCCCCAGATC-3′
	AS	5′-AGCGGCCGCTTAGTCTTCATCCCAGGGGAAGGTCGGCATC-3′
2. NH_3 (8)	S	5′-ACGCGTCGACATCCCGGGCTTGTGGGACACC-3′
	AS	5′-GCGGCCGCTTATTATGAGGGTCGTGGGTGCGG-3′
3. NH_3 (18)	S	5′-ACGCGTCGACATCTCGGAAATCAGCCAGAAG-3′
	AS	5′-GCGGCCGCTTATTATGAGGGTCGTGGGTGCGG-3′
4. NH_3 (23)	S	5′-ACGCGTCGACAGCCAGAAGTTGCCAGGTGAATAC-3′
	AS	5′-GCGGCCGCTTATTATGAGGGTCGTGGGTGCGG-3′
5. D19A	S	5′-GGACACCTATGAAGATGCCATCTCGGAAATCAGC-3′
	AS	5′-GCTGATTTCCGAGATGGCATCTTCATAGGTGTCC-3′
6. I20A	S	5′-GACACCTATGAAGATGACGCCTCGGAAATCAGCCAG-3′
	AS	5′-CTGGCTGATTTCCGAGGCGTCATCTTCATAGGTGTC-3′
7. I20E	S	5′-GACACCTATGAAGATGACGAATCGGAAATCAGCCAG-3′
	S	5′-CTGGCTGATTTCCGATTCGTCATCTTCATAGGTGTC-3′
8. I20Q	S	5′-GACACCTATGAAGATGACCAATCGGAAATCAGCCAG-3′
	AS	5′-CTGGCTGATTTCCGATTGGTCATCTTCATAGGTGTC-3′
9. I20L	S	5′-GACACCTATGAAGATGACCTCTCGGAAATCAGCCAG-3′
	AS	5′-CTGGCTGATTTCCGAGAGGTCATCTTCATAGGTGTC-3′
10. I20M	S	5′-GACACCTATGAAGATGACATGTCGGAAATCAGCCAG-3′
	AS	5′-CTGGCTGATTTCCGACATGTCATCTTCATAGGTGTC-3′
11. I20K	S	5′-GACACCTATGAAGATGACAAATCGGAAATCAGCCAG-3′
	AS	5′-CTGGCTGATTTCCGATTTGTCATCTTCATAGGTGTC-3′
12. S21A	S	5′-CACCTATGAAGATGACATCGCGGAAATCAGCCAG-3′
	AS	5′-CTGGCTGATTTCCGCGATGTCATCTTCATAGGTG-3′
13. E22A	S	5′-GAAGATGACATCTCGGCAATCAGCCAGAAGTTG-3′
	AS	5′-CAACTTCTGGCTGATTGCCGAGATGTCATCTTC-3′
14. I23A	S	5′-GATGACATCTCGGAAGCCAGCCAGAAGTTGCCAGG-3′
	AS	5′-CCTGGCAACTTCTGGCTGGCTTCCGAGATGTCATC-3′
15. I23E	S	5′-GATGACATCTCGGAAGAAAGCCAGAAGTTGCCAGG-3′
	AS	5′-CCTGGCAACTTCTGGCTTTCTTCCGAGATGTCATC-3′
16. I23Q	S	5′-GATGACATCTCGGAACAAAGCCAGAAGTTGCCAGG-3′
	AS	5′-CCTGGCAACTTCTGGCTTTGTTCCGAGATGTCATC-3′
17. I23L	S	5′-GATGACATCTCGGAACTCAGCCAGAAGTTGCCAGG-3′
	AS	5′-CCTGGCAACTTCTGGCTGAGTTCCGAGATGTCATC-3′
18. I23M	S	5′-GATGACATCTCGGAAATGAGCCAGAAGTTGCCAGG-3′
	AS	5′-CCTGGCAACTTCTGGCTCATTTCCGAGATGTCATC-3′
19. I23K	S	5′-GATGACATCTCGGAAAAAAGCCAGAAGTTGCCAGG-3′
	AS	5′-CCTGGCAACTTCTGGCTTTTTTCCGAGATGTCATC-3′

were collected and analyzed by SDS-polyacrylamide gel electrophoresis, and cleavage proteins were visualized with the GelCode Blue Stain reagent from Pierce. Protein concentrations were determined using the BCA protein assay kit (Pierce) and bovine serum albumin as a standard.

Construction of Truncated Proteins—Amino- and carboxyl-terminal truncations were generated using PCR. *PfuTurbo* Hotstart DNA polymerase (Stratagene, La Jolla, CA) and primers as described in Table I were used for PCR under the following conditions: predenaturing at 95 °C for 2 min followed by 18 cycles of denaturing at 95 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 6 min. PCR products were digested with *Sal*I and *Not*I and ligated into *Sal*I/*Not*I-digested pGEX-6P-3 vector directly. Sequencing identified appropriate clones.

Site-selected Mutagenesis—Mutations involving specific amino acid residues of the amino-terminal region of SULT2B1b were generated using QuikChange XL site-directed mutagenesis kit according to the manufacturer's instructions (Stratagene). Briefly, 25 ng of pGEX-SULT2B1b were used as a template and mutated nucleotide primers as described in Table I. PCR conditions were: predenaturing at 95 °C for 2 min, followed by 18 cycles of denaturing at 95 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 6 min. After digestion with *Dpn*I, 2 μl of PCR product were used to transform XL10-Gold competent cells provided with the kit. Sequencing identified appropriate clones.

Sulfotransferase Assay—Sulfotransferase activity was determined using radiolabeled cholesterol and pregnenolone. Twenty-μl reaction volumes contained the same concentration of either an overexpressed and purified wild type protein or a mutant construct (4.0 μg/tube, SULT2B1a, and 0.4 μg/tube, SULT2B1b for cholesterol (5 μM); 0.4 μg/tube, SULT2B1a, and 0.4 μg/tube, SULT2B1b for pregnenolone (20 μM)) in 0.1 mM Tris-HCl buffer (pH 7.5) containing 5 mM $MgCl_2$, 0.2 mM 2-hydroxypropyl-β-cyclodextrin, and 4% ethanol (v/v). Reactions were carried out at 37 °C for 5 min and stopped by placing tubes in boiling water for 5 min. To each reaction tube, 10 μl of 5 mg/ml of either cholesterol sulfate or pregnenolone sulfate in 10% 2-hydroxypropyl-β-cyclodextrin were added as a carrier, and 5 μl were applied to a TLC plate, which was developed using a solvent system consisting of chloroform/methanol/acetone/acetic acid/water (8:2:4:2:1). After drying, the TLC plate was exposed to I_2 vapor to visualize the steroid/sterol sulfate spots, which were excised, and the radioactivity was determined by liquid scintillation spectrometry. For the kinetic analyses, assays were similarly carried out using the following purified protein preparation: SULT2B1a, 4.0 μg, and SULT2B1b, 0.4 μg in the cholesterol assay, and SULT2B1a, 0.4 μg, SULT2B1b, 0.4 μg in the pregnenolone assay.

RESULTS

SULT2B1a and SULT2B1b Substrate Preferences—The wild type protein preparations used throughout this investigation as well as all mutated protein preparations were judged to be >90% pure based on analysis by SDS-PAGE and protein staining (data not presented). A steady state analysis of the

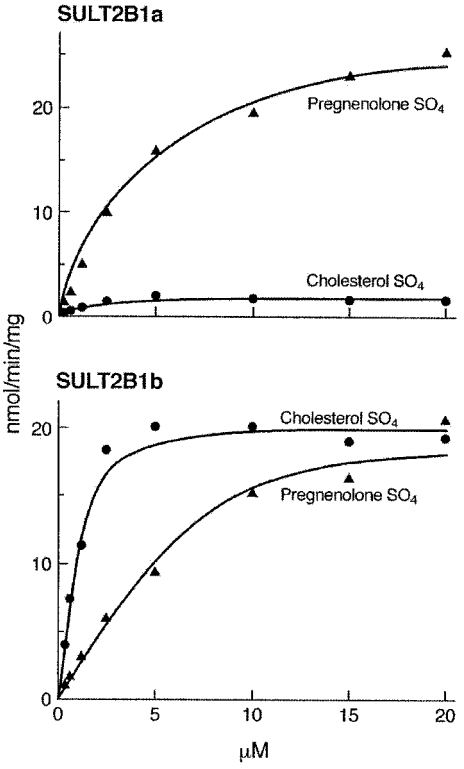

FIG. 1. **Kinetic analyses of SULT2B1a and SULT2B1b employing cholesterol and pregnenolone as substrates.** Proteins were overexpressed in bacteria as GST fusion proteins, cleaved, and affinity-purified, and the sulfotransferase assays were carried out as described under "Experimental Procedures."

SULT2B1 isoforms using cholesterol and pregnenolone as substrates is depicted in Fig. 1. The SULT2B1a isoform avidly sulfonates pregnenolone, whereas sulfonation of cholesterol is minimal. In contrast, the SULT2B1b isoform preferentially sulfonates cholesterol, reaching a maximum at a substrate concentration of 5 μM. SULT2B1b, however, also sulfonates pregnenolone, although a steady state is not reached until the substrate concentration reaches ~20 μM, at which point the sulfonation of pregnenolone essentially equals that of cholesterol. Nevertheless, the greater efficiency of the SULT2B1b isoform for cholesterol is revealed by a k_{cat}/K_m ratio of 11.1 ± 1.2 $M^{-1}S^{-1} \times 10^3$ for cholesterol *versus* 1.6 ± 0.4 $M^{-1}S^{-1} \times 10^3$ for pregnenolone. Notably, neither SULT2B1 isoform sulfonates dehydroepiandrosterone efficiently (data not presented).

Influence of the Amino- and Carboxyl-terminal Ends of the SULT2B1 Isoforms on Catalysis—Removal of 53 amino acids from the carboxyl-terminal end that is common to both SULT2B1a and SULT2B1b (compare Fig. 2) does not significantly reduce the ability of either SULT2B1a to sulfonate pregnenolone or SULT2B1b to sulfonate cholesterol (Fig. 3). Likewise, removal of 8 amino acids from the amino-terminal end that is unique to the SULT2B1a isoform (compare Fig. 2) does not significantly alter pregnenolone sulfotransferase activity (Fig. 3). On the other hand, removal of 23 amino acids from the amino-terminal end that is unique to the SULT2B1b isoform (compare Fig. 2) results in an almost complete loss in cholesterol sulfotransferase activity (Fig. 3).

Cholesterol Sulfotransferase Activity Following Progressive Truncation of the Amino-terminal End of SULT2B1b—In contrast to the removal of the first 23 amino acids from the amino-terminal end of SULT2B1b, which leads to a loss in catalysis, cholesterol sulfotransferase activity is essentially unaffected after removal of the first 8 amino acids (Fig. 4). Although removal of the first 18 amino acids results in an apparent increase in catalytic activity (Fig. 4), the K_m value of 1.1 μM for the −18 amino-terminal truncated protein is not significantly different from the K_m value of 1.2 μM for wild type.

Effect of Amino Acid Substitutions in the Amino-terminal End of SULT2B1b on Cholesterol Sulfotransferase Activity—The loss in catalytic activity that occurs between the −18 and −23 amino-terminal truncations of SULT2B1b clearly indicates that the sequence of *DISEI* between residues 18 and 24 (compare Fig. 2) is crucial for functionality. Therefore, this sequence was analyzed by alanine scanning (Fig. 5). D19A, S21A, and E22A substitutions result in levels of cholesterol sulfotransferase activity that are higher than that of wild type, although the K_m values for these substitutions are, respectively, 1.7, 1.8, and 1.9 μM, they are not significantly different from the K_m value of 1.8 μM for wild type. In contrast to the aforementioned substitutions, both the I20A and I23A substitutions result in a nearly complete loss of cholesterol sulfotransferase activity (Fig. 5). Interestingly, the latter substitutions have no effect on the ability of this isoform to sulfonate pregnenolone (compare Fig. 1) (data not shown).

This phenomenon was further examined by determining the effect of substituting a variety of amino acids other than alanine for the isoleucines at positions 20 and 23. Substitutions were carried out separately for each isoleucine residue with results that are essentially the same (Fig. 6). Substitutions involving a negatively charged (glutamic acid) and a positively charged (lysine) amino acid, as well as a substitution involving a polar but uncharged amino acid with a side chain similar in size to isoleucine (glutamine), result in loss of catalytic activity similar to the alanine substitutions (Fig. 6). On the other hand, use of a conservative substitution (leucine) results in 80% (residue 20) and 100% (residue 23) retention of catalytic activity (Fig. 6). Use of methionine, a somewhat less conservative substitution for isoleucine than leucine but with a side chain similar in size, results in a partial retention (~30% at residue 20 and 60% at residue 23) in cholesterol sulfotransferase activity (Fig. 6). Importantly, the K_m values of 1.4 μM for wild type and 1.7 μM for I20L, 1.7 μM for I23L, 1.4 μM for I20M, and 1.3 μM for I23M are not significantly different.

DISCUSSION

Overall, the SULT2A1 and SULT2B1 isozymes are ~37% identical at the amino acid level. If, however, the extended amino- and carboxyl-terminal ends of the SULT2B1 isoforms are excluded, identities increase to ~48%. Interestingly, all previously cloned members of the mammalian cytosolic sulfotransferase superfamily, *i.e.* estrogen and phenol sulfotransferases as well as hydroxysteroid sulfotransferases, have sizes that range from 282 to 295 amino acids, whereas SULT2B1a and SULT2B1b consist of 350 and 365 amino acids, respectively. Nonetheless, the extended amino- and carboxyl-terminal ends of the SULT2B1 isoforms aside, there remains a significant structural similarity between the SULT2A1 and SULT2B1 isozymes in their core regions. Most notably, a PSB loop (a P-loop motif found at phosphate-binding sites of nucleotide-binding proteins) and specific amino acid residues important in protein-cofactor interaction of cytosolic sulfotransferases (15, 16) are with but one exception completely conserved (compare Fig. 2). Furthermore, regions interacting with the 5′- (5′-PB) and 3′- (3′-PB) phosphate groups of PAPS are highly conserved. Of particular structural interest is a conserved lysine at residue 55 in SULT2B1a and residue 70 in SULT2B1b along with a conserved serine at residue 140 in SULT2B1a and residue 155 in SULT2B1b. The significance of this is that in the crystal structure of human estrogen sulfotransferase-PAPS complex, the side chain nitrogen of a compa-

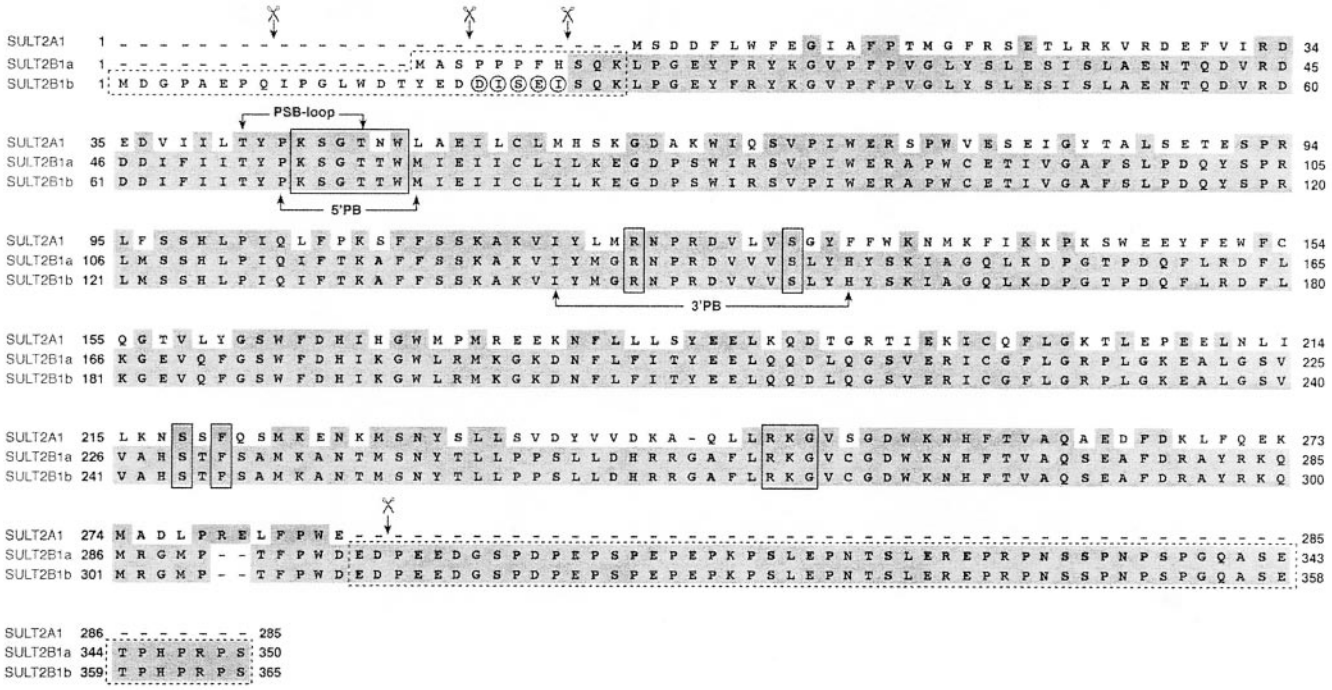

FIG. 2. **Amino acid alignment of the three human hydroxysteroid sulfotransferases SULT2A1, SULT2B1a, and SULT2B1b.** *Shading* indicates areas of identity. *Boxes* made with *dashed lines* outline the extended amino- and carboxyl-terminal ends of SULT2B1a and SULT2B1b. *Boxes* made with *solid lines* indicate conserved amino acid residues involved in cofactor interaction. The nucleotide-binding PSB loop and the 5′- and 3′-phosphate-binding motifs (5′-*PB* and 3′-*PB*) are delineated by *arrows*. *Scissors symbols* indicate regions subject to deletion, and the *encircled* amino acids in the amino-terminal region of SULT2B1b represent residues subject to site-directed mutagenesis.

rable lysine, is thought to interact with the side chain hydroxyl of a comparable serine, indicating that the serine plays an important role in regulating side chain interaction of the catalytic lysine with the bridging oxygen between the 5′-phosphate and sulfate of PAPS (17). The core regions of the SULT2B1 isoforms thus contain residues that are highly conserved in all cytosolic sulfotransferases, residues now considered to be involved in interaction with the PAPS cofactor. This leaves the extended amino- and carboxyl-terminal ends as the most outstanding feature of the SULT2B1 isoforms and the principal structural distinction between them and the prototypical SULT2A1 isozyme as well as other members of the large cytosolic sulfotransferase superfamily.

The functional significance of the extended carboxyl-terminal end of the SULT2B1 isoforms is not known. One speculation is that this region, which is enriched in prolines, might play a role in protein-protein interactions (10). Regardless, it is notable that the relatively long carboxyl-terminal extension (53 amino acids), which is structurally common to both SULT2B1 isoforms, can be removed without producing a significant change in the catalytic behavior of either isoform. On the other hand, removal of the unique amino-terminal ends, which distinguish the SULT2B1 isoforms, produces interesting results. Firstly, removal of the unique amino-terminal end of SULT2B1a consisting of 8 amino acids does not significantly alter catalytic activity, whereas removal of the unique amino-terminal end of SULTT2B1b consisting of 23 amino acids has a profound influence on cholesterol catalysis. The latter finding notwithstanding, however, the first 18 amino acids of the SULT2B1b amino-terminal region are not essential for catalytic activity, which leads to the second point. That is, the 5-amino acid segment between residues 18 and 24 from the amino terminus of SULT2B1b is the crucial structural feature of this isoform that is essential for full cholesterol sulfotransferase activity. Thirdly, the isoleucines within this 5-amino acid segment of SULT2B1b are critically involved in catalytic behavior. Additionally, based on the testing of a variety of amino acid substitutions, it appears that only a hydrophobic amino acid with a side chain of optimal size can effectively function at these positions. That is, alanine does not work, whereas leucine works essentially as well as isoleucine; furthermore, methionine, although less effective than isoleucine, is also able to sustain significant enzymatic activity. Both of the latter substitutions, interestingly, produce enzymes with K_m values similar to the wild type protein, denoting the effectiveness of these conservative substitutions. Conversely, charged and polar amino acid substitutions at either of the isoleucine positions are unable to sustain enzymatic activity. Interestingly, in the SULT2B1a isoform, the amino acid residues at positions comparable to the isoleucines at positions 20 and 23 in SULT2B1b, are proline and histidine, respectively (compare Fig. 2).

The unique amino-terminal end of SULT2B1b is clearly responsible for the ability of this isoform to sulfonate cholesterol. Conversely, the unique amino-terminal end of SULT2B1a is not required for this isoform to sulfonate pregnenolone, and thus its functional significance is not presently appreciated. The importance of the amino-terminal end of SULT2B1b for cholesterol specificity as well as catalysis is further emphasized by the fact that, in face of the various truncations and amino acid substitutions carried out on this isoform, which profoundly influence cholesterol reactivity, there is no adverse effect of any of these machinations on the ability of SULT2B1b to sulfonate pregnenolone. In essence, with the unique amino-terminal end of SULT2B1b removed, it behaves essentially like SULT2B1a. It seems curious that the unique amino terminus of one SULT2B1 isoform is absolutely essential for functionality, whereas a related structural characteristic in the other isoform is not required for it to normally function. Of course, this paradox or mystery should largely disappear once the three-dimensional structures have been solved.

The gene for human *SULT2B1* consists of an exon 1B, an

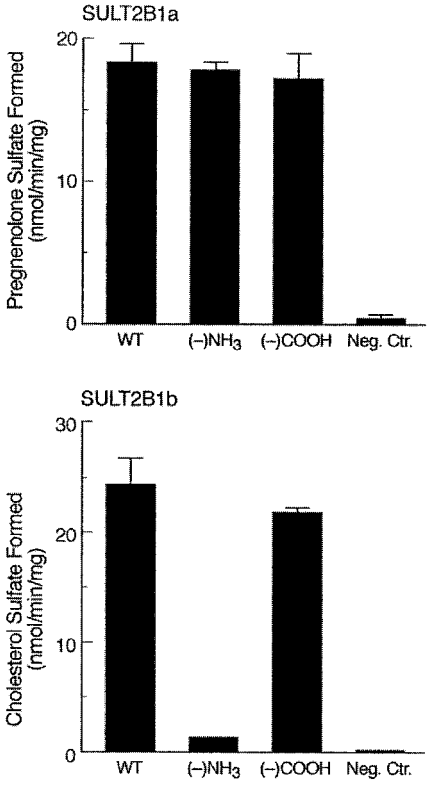

FIG. 3. **Sulfotransferase activity after truncation of the amino- and carboxyl-terminal ends of the SULT2B1 isoforms.** Wild type (*WT*) of SULT2B1a and SULT2B1b and constructs of each isoform lacking either the unique amino-terminal end (*(−)NH$_3$*) or 52 amino acids from the common carboxyl-terminal end (*(−)COOH*) (compare Fig. 2) were prepared as GST fusion proteins, cleaved, affinity-purified, and assayed for either pregnenolone (SULT2B1a) or cholesterol (SULT2B1b) sulfotransferase activity as described under "Experimental Procedures" using 20 and 5 μM, respectively, of each substrate. Negative control (*Neg. Ctr.*) indicates the result when a wild type preparation is assayed in the absence of the PAPS cofactor. The *top* of each column represents the mean of five replicates, and *error bars* indicate the standard deviation.

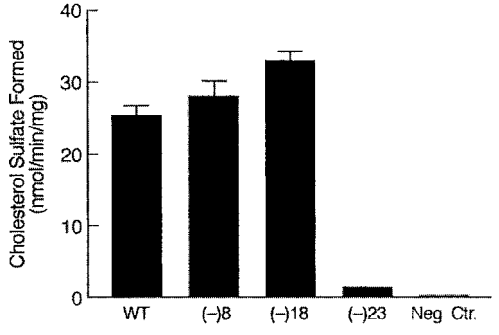

FIG. 4. **Cholesterol sulfotransferase activity of SULT2B1b after progressive truncations of the amino-terminal end.** Wild type (*WT*) SULT2B1b and constructs of SULT2B1b in which the amino-terminal end has been shortened by 8, 18, and 23 amino acids (compare Fig. 2) were prepared as GST fusion proteins, cleaved, affinity-purified, and assayed for cholesterol sulfotransferase activity using 5 μM substrate concentration as described under "Experimental Procedures." Negative control (*Neg. Ctr.*) indicates the result when the wild type preparation is assayed in the absence of the PAPS cofactor. The *top* of each column represents the mean of five replicates, and *error bars* indicate the standard deviation.

exon 1A, and exons 2–6: the SULT2B1a isoform is encoded by exons 1A and exons 2–6, and the SULT2B1b isoform is encoded by exon 1B, the final 143 nucleotides of exon 1A, plus exons 2–6 (1). Exon 1B is composed of a 5′-untranslated region consisting

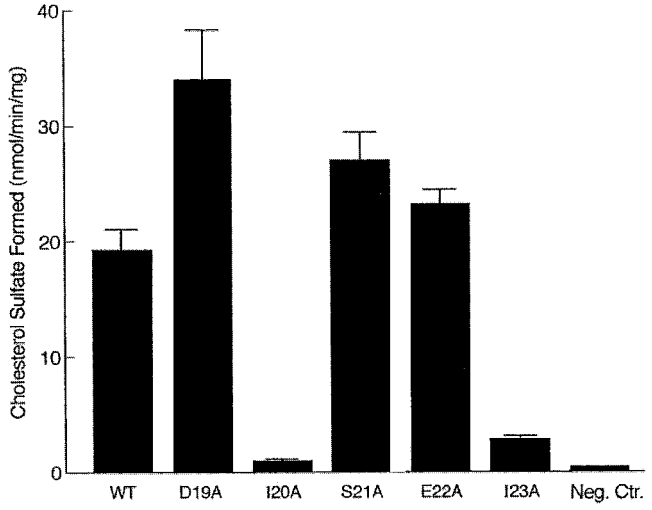

FIG. 5. **Sulfotransferase activity of SULT2B1b after site-directed mutagenesis involving the amino-terminal region.** Wild type (*WT*) SULT2B1b and constructs of SULT2B1b in which a *DISEI* sequence in the amino-terminal region (compare Fig. 2) has been subject to alanine scanning were prepared as GST fusion proteins, cleaved, affinity-purified, and assayed for cholesterol sulfotransferase activity using 5 μM substrate concentration as described under "Experimental Procedures." Negative control (*Neg. Ctr.*) indicates the result when the wild type preparation is assayed in the absence of the PAPS cofactor. The *top* of each column represents the mean of five replicates, and *error bars* indicate the standard deviation.

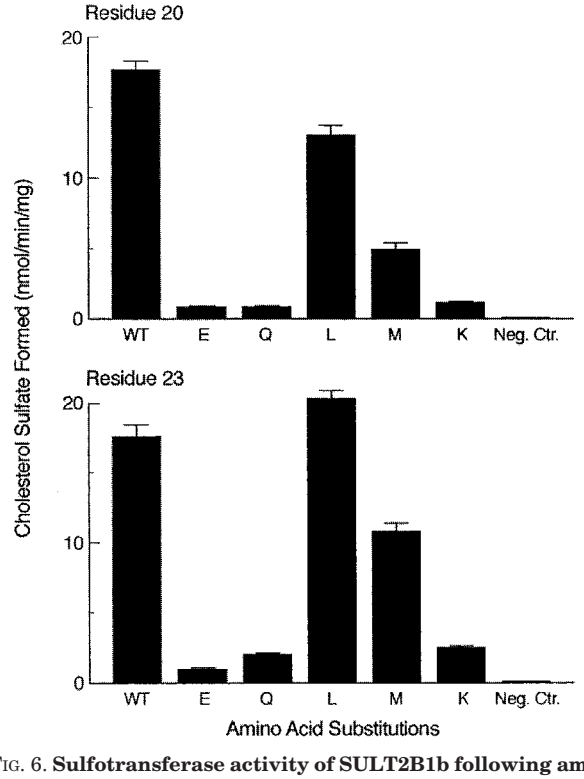

FIG. 6. **Sulfotransferase activity of SULT2B1b following amino acid substitutions of the *DISEI* sequence in the amino-terminal region.** Wild type (*WT*) SULT2B1b and constructs of SULT2B1b in which the isoleucines at positions 20 and 23 from the amino terminus, *i.e.* within the *DISEI* sequence (compare Fig. 2), have been separately replaced with one of the following amino acids, as indicated in the figure: glutamic acid, glutamine, leucine, methionine, or lysine. Proteins were prepared as GST fusion proteins, cleaved, affinity-purified, and assayed for cholesterol sulfotransferase activity using 5 μM substrate concentration as described under "Experimental Procedures." Negative control (*Neg. Ctr.*) indicates the result when the *WT* preparation is assayed in the absence of the PAPS cofactor. The *top* of each column represents the mean of five replicates, and *error bars* indicate the standard deviation.

of 129 nucleotides and the coding region for the first 23 amino acids of SULT2B1b, which represents the entire amino-terminal region that is unique to this isoform (1). Exon 1A, on the other hand, in addition to a 179-nucleotide 5′-untranslated region, encodes for the first 56 amino acids of SULT2B1a, of which only the first 8 amino acids are unique to this isoform (1). Thus, when the gene for human *SULT2B1* employs exon 1B, cholesterol sulfotransferase is synthesized, whereas when the gene uses exon 1A, pregnenolone sulfotransferase is produced. This raises an interesting question regarding the biological significance of this differential expression?

In considering feasible physiological implications of the differential expression of the gene for human *SULT2B1*, two organ systems loom as particularly attractive, *i.e.* skin and brain. It is now recognized that cholesterol sulfate plays an essential role in skin development and creation of the barrier (18–21). Furthermore, as determined by real-time PCR, expression of the human SULT2B1b isoform, which we now recognize as a cholesterol sulfotransferase, is higher in skin than in any other organ with the possible exception of the placenta and the prostate.[2] Although expression of the human SULT2B1 isoforms in the adult central nervous system remains to be adequately examined, the human fetal brain, as determined by reverse transcription-PCR, appears to express only the SULT2B1a isoform (12). Interestingly, the mouse SULT2B1a ortholog appears to be almost exclusively expressed in brain tissue.[3] The significance of these findings is that sulfated pregnenolone, which is produced most efficiently by the action of the SULT2B1a isoform, is now appreciated as an essential neurosteroid (22–25).

[2] H. Fuda, Y. C. Lee, C. Shimizu, N. B. Javitt, and C. A. Strott, unpublished observations.

[3] C. Shimizu, H. Fuda, H. Yanai, and C. A. Strott, unpublished observations.

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